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# Development and Validation of Spectrofluorometric Method for Dapagliflozin Estimation in Bulk & Formulation

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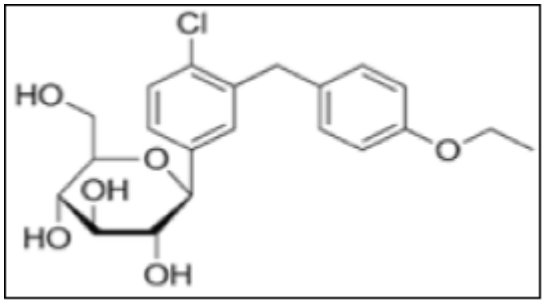
**Abstract:** ***Objective: Developed and validated accurate spectrofluorometric method for Dapagliflozin estimation in pharmaceutical dosage form. Method: Dapagliflozin showed good fluorescence intensity in methanol so it was selected as a solvent for estimation of Dapagliflozin. The optimized excitation (λex) and emission (λem) wavelength were 277 nm and 604 nm respectively. Results: Linear regression analysis data for the calibration plots showed a good linear relationship with $R^2$ 0.999 for Dapagliflozin in the concentration range of 2-10 µg/mL. The repeatability of the method in terms of % RSD was found to be 0.68 %. The intraday and interday precision of the method in terms of %RSD was found to be 0.74-1.34% and 0.75-1.83%, respectively. LOD for Dapagliflozin was found to be 0.15 µg/mL, while LOQ was found to be 0.46 µg/mL. %Recovery of Dapagliflozin was found in the range of 99.40-99.76%. The method was successfully applied to pharmaceutical dosage for analysis of Dapagliflozin. The assay result was found to be 99.90%. The developed method was validated in terms of linearity, precision, accuracy, limit of detection and quantification, robustness as per International Conference on Harmonization Q2 (R1) guidelines. Conclusion: This method is rapid, accurate, and simple, making it suitable for routine Dapagliflozin analysis in quality control labs without separation.***

**Keywords:** Spectrofluorimetric, Fluorescence, Excitation, Anti Diabetic and Emission.

## 1. Introduction[1-5]

Dapagliflozin, a once-daily oral antidiabetic, is a selective inhibitor of the sodium glucose co-transporter type 2 (SGLT2), in development with Bristol-Myers Squibb and AstraZeneca for the treatment of type 2 diabetes mellitus.Type 2 diabetes is a chronic disease. It is characterized by high levels of sugar in the blood. Type 2 diabetes is also called type 2 diabetes mellitus and adult-onset diabetes. That's because it used to start almost always in middle- and late-adulthood. However, more and more children and teens are developing this condition. Type 2 diabetes is much more common than type 1 diabetes, and is really a different disease. But it shares with type 1 diabetes high blood sugar levels, and the complications of high blood sugar. During digestion, food is broken down into basic components. Carbohydrates are broken down into simple sugars, primarily glucose. Glucose is a critically important source of energy for the body's cells. To provide energy to the cells, glucose needs to leave the blood and get inside the cells. Insulin traveling in the blood signals the cells to take up glucose. Insulin is a hormone produced by the pancreas. The pancreas is an organ in the abdomen. When levels of glucose in the blood rise (for example, after a meal), the pancreas produces more insulin.

Chemically it is (2S,3R,4R,5S,6R)-2-[4-chloro-3-[(4-ethoxyphenyl)methyl]phenyl]-6-(hydroxymethyl) oxane-3,4,5-triol. Dapagliflozin works by helping the kidneys get rid of glucose from your bloodstream. Dapagliflozin is used together with diet and exercise to improve blood sugar control in adults with type 2 diabetes mellitus. Dapagliflozin is not for treating type 1 diabetes. SGLT2 is a low affinity, high-capacity transporter located in the brush-border membrane of the early segment of the proximal tubule of the kidney.This transporter is responsible for 90% of the glucose reabsorbed by the kidneys. SGLT2 inhibitors maintain blood glucose levels by regulating the reabsorption of filtered glucose. Blocking this transporter mechanism causes blood glucose to be eliminated through the urine.Dapagliflozin works by helping the kidneys get rid of glucose from your bloodstream.




**Chemical structure of Dapagliflozin**

The literature survey reveals that Dapagliflozin can be estimated by various methods like UV-spectrophotometric[6-8], by HPLC [9-11] and by HPTLC [12]individually or with other drugs in bulk drugs and in human plasma. However, there is no any Spectrofluorimetric method has been reported for the estimation of Dapagliflozin. The present work describes the fast, economic, specific and selective

Spectrofluorimetric method for the estimation of Dapagliflozin in pharmaceutical dosage forms. There were numbers of trial performed in different solvent like 1N HCl, 1N NaOH, Methanol and distill water. Among the all methanol was shown good intensity and no interference compared to other solvent so proposed method is direct and based on the measurement fluorescence intensity of Dapagliflozin in methanol.

## 2. Materials and Methods

Pharmaceutical grade of Dapagliflozin was kindly gifted by Glenmark Pharmaceuticals (Ankleshwar, India). A tablet formulation was purchased from the local market (Forxiga containing Dapagliflozin 10 mg). All the reagents used in this method were of analytical. Methanol which is used as a solvent was purchased from Rankem, Avantor Performance Materials India Limited, Thane, IndiaSpectrofluorimetric analysis was performed on Shimadzu Spectrofluorimeter with xenon discharge lamp (20KW), two automatic monochromators, Photomultiplier tube as detector; Software (RF-5301 PC Software RFPC, VERSION 3) and quartz cuvette was used. All weights were taken on electronic analytical balance Reptech RA Series.

**Preparation of solutions: -**

- Preparation of standard stock solution of Dapagliflozin (DPG): An accurately weighed quantity of about 10 mg of DPG was transferred in a 10 mL volumetric flask, dissolved in sufficient quantity of methanol, sonicated for 30 min and diluted to 10 mL with the same solvent so as to get the concentration of 1000 µg/mL.
- Preparation of working standard solution of DPG: Aliquot of 1 Ml from standard stock solution was transferred into 10 Ml volumetric flask and diluted up to the mark with methanol to get the concentration of 100 µg/Ml.
- **Procedure for the determination of the excitation and** emission wavelength: From the working standard solutions containing 100 µg/mL of DPG dilutions were made to prepare solution having concentration 20 µg/mL of DPG and scanned for the excitation and emission wavelength using spectrofluorometer. DPG resulting solution was scanned for the emission spectrum using excitation wavelength.

**For Calibration curve:**

From the working standard solution 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL,10 mL were pipetted into 10 mL volumetric flasks and volumes were made up to the mark with methanol to produce the concentrations ranging from 2-10 µg/mL respectively.

**Validation Method**[13,14]

The developed method was validated as per the International Council of Harmonization (ICH) guidelines with respect to linearity, precision, Robustness, the limit of detection and limit of quantification.

**Linearity**

From the working standard solutions containing 100 µg/mL of DPG, dilutions were made to prepare a range of standard solutions having different concentrations of DPG (2-10 µg/mL). The intensity of emission was measured at 604 nm when excited at 277 nm. The calibration curves of respective concentration versus fluoresces intensity were plotted and correlation coefficient and regression line equations were computed.

**Precision**

**Repeatability**

Repeatability of the method was determined by performing seven replicates analysis of the working solution (6 µg/mL).

**Intra-Day and Inter-Day Precision**

Inter-day precision (%RSD) was determined by analysing the working standard solution of DPG over the entire calibration range (2-10 µg/mL) on 3 different days.

Intra-day precision (%RSD) was determined by analysing the working standard solution of DPG over the entire calibration range (2-10 µg/mL) for 3 times in a day.

**Limit of Detection and Limit of Quantitation**

The limits of detection of the developed method were calculated from the standard deviation of the intercepts and mean slope of the calibration curves of DPG using the equation given below:

$$\mathbf{LOD = 3.3 \times SD/S}$$

Where, SD is the standard deviation of the Y-intercepts of the seven calibration curves and S is the mean slope of the seven calibration curves.

The limits of quantitation of the developed method were calculated from the standard deviation of the intercepts and mean slope of the calibration curves of DPG using the equation given below:

$$\mathbf{LOQ = 10 \times SD/S}$$

Where, SD is the standard deviation of the Y-intercepts of the seven calibration curves and S is the mean slope of the seven calibration curves.

**Accuracy**

The accuracy of the developed method was determined by recovery studies at three levels (80%, 100% and 120%) by the standard addition method.

Recovery study was carried out by spiking three different known amounts (8 mg, 10 mg and 12 mg) of the standard drug to the pharmaceutical dosage form (10 mg). Three numbers of 100 mL volumetric flasks were taken. Standard DPG 8 mg, 10 mg and 12 mg were spiked in first, second, and third volumetric flasks, respectively. All three flasks were filled to about 80% with methanol, sonicated for 30 minutes and diluted up to the mark with methanol.

These solutions were filtered through Whatman filter paper individually. From each filtrate, 1 mL of each was diluted up to 10 mL with methanol individually. Aliquot of 1 mL of each above solution was diluted up to 10 mL with methanol. Further aliquot of 1 mL of each above solution was diluted up to 10 mL with methanol. From the calibration curve, the

amount of DPG recovered was calculated and % recovery was determined. The fluoresces intensity of each solution was measured emission wavelength at 604 nm. From the calibration curve, the amount of DPG recovered was calculated and % recovery was determined.

**Table:1 Preparation of solutions for accuracy study**

| Level | DPG (mg) from formulation | Spiked DPG amount (mg) | Concentration of DPG solution (µg/mL) |
|---|---|---|---|
| 80% | 10 | 8 | 5.4 |
| 100% | 10 | 10 | 6 |
| 120% | 10 | 12 | 6.6 |

**Robustness**

Robustness was determined by the analysis of solutions (2-10 µg/mL) at different excitation wavelengths (± 2 nm). Solutions were scanned at different scan speeds (slow, medium and fast). The % RSD of the results was used to evaluate the method robustness.

**Assay of pharmaceutical dosage form**

The pharmaceutical dosage form was analysed using the developed method. Accurately weighed amount from formulation equivalent to 10 mg DPG was transferred to 100 mL volumetric flask, dissolved in few mL methanol then sonicated for 30 min and diluted up to the mark with methanol and filtered through Whatman filter. Aliquot 1 mL from the filtrate was transferred to 10 mL volumetric flask and diluted up to the mark with methanol. Aliquot 1 mL from the filtrate was transferred to 10 mL volumetric flask and diluted up to the mark with methanol. Aliquot 3mL from the above solution was transferred into 10 mL volumetric flask and diluted up to mark with methanol. The intensity of the resulting solution was measured at emission wavelength 604 nm. The amount of DPG present in the sample solution was determined by fitting area values of the corresponding peak into the equation representing the calibration curve of DPG and % labelled claim of the drug was computed.

## 3. Results and Discussion

**Determination of Excitation and Emission Wavelength**

The Excitation and Emission wavelength for the DPG was found to be 277 nm and 604 nm respectively in figure:1

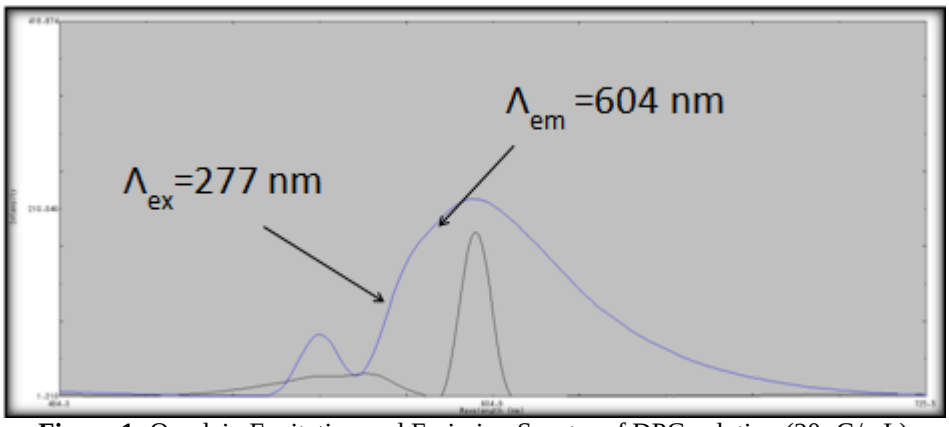



**Figure 1:** Overlain Excitation and Emission Spectra of DPG solution (20µG/mL)

**Preparation calibration curve**

A calibration curve was prepared in the range of 2-10 µg/mL for DPG. The Fluorescence intensity of DPG increases linearly with concentration. Data of the calibration curve is shown in Table 2. Calibration curve of DPG is depicted in figure:2 & 3.

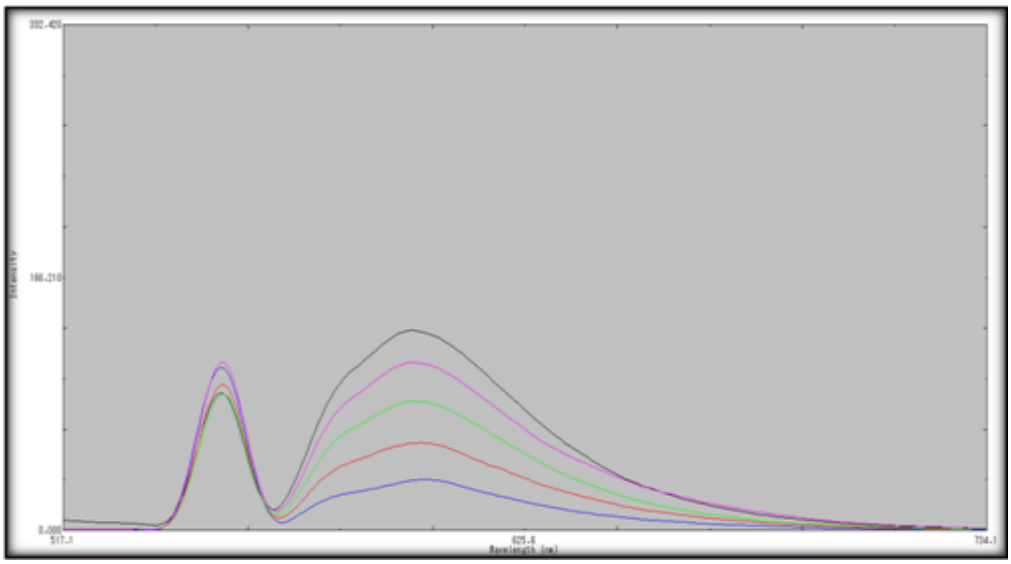


**Figure 2:** Overlain spectra of DPG in Methanol (2-10 µg/mL)

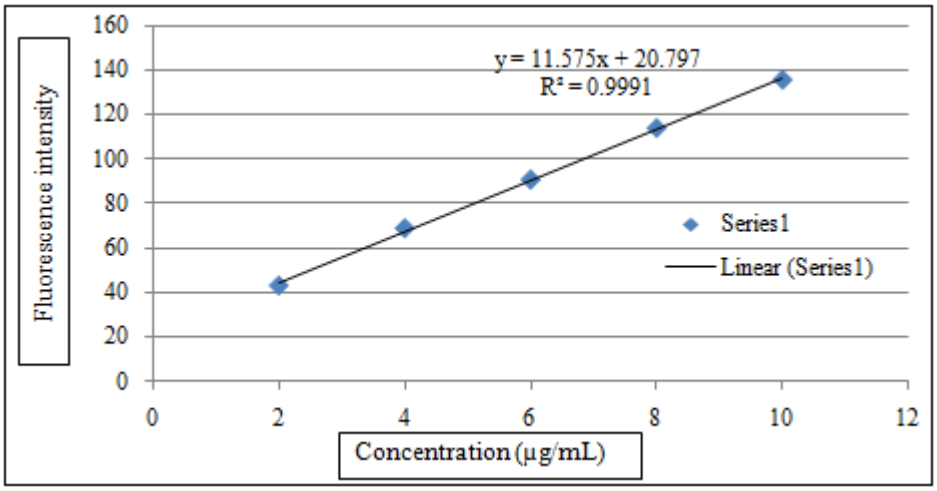



**Figure 3:** Calibration curve for DPG

**Table 2:** Calibration data for DPG

| Concentration (100-500 ng/mL) | Fluorescence intensity |
|---|---|
| 2 | 42.706 |
| 4 | 68.686 |
| 6 | 90..636 |
| 8 | 113.9802 |
| 10 | 135.813 |

## Validation Method

### Linearity and Range

Representative calibration curve of DPG was obtained by plotting the mean Fluorescence intensity of DPG against concentration over the range 2-10 µg/mL (n=7). It was found to be linear in the above-mentioned range with a coefficient of regression 0.999. The %RSD for each level of DPG was found to be in the range of 0.5-1.31%. The average linear regressed equation for the calibration curve was y = 11.572x+20.798. Linearity data is depicted in Table:3 &4.

**Table 3:** Calibration data for DPG

| Concentration (ng/mL) | Florescence intensity (mean ± SD) (n=7) | % RSD |
|---|---|---|
| 100 | 42.617±.559 | 1.31 |
| 200 | 68.662±.664 | 0.96 |
| 300 | 90.453±.621 | 0.68 |
| 400 | 113.537±.869 | 0.76 |
| 500 | 135.909±.729 | 0.53 |

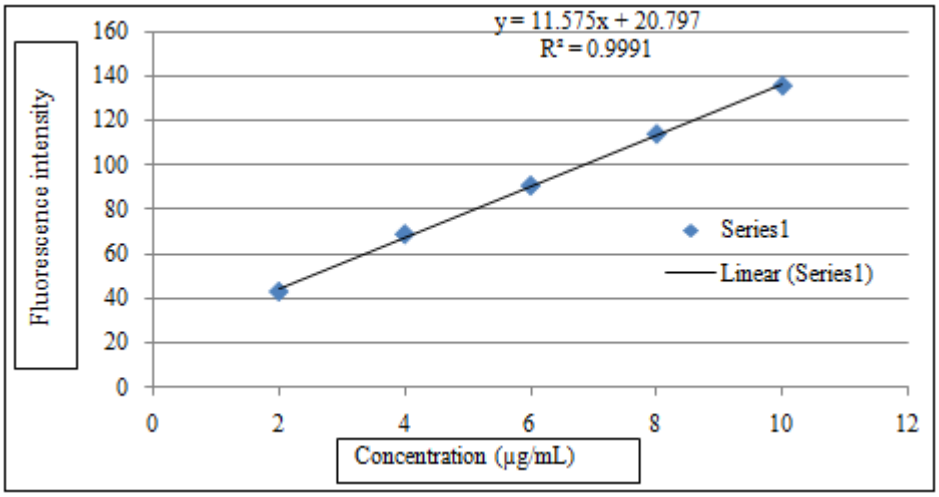



**Figure 4:** Calibration curve for DPG

**Table 4:** Summary of linearity data for DPG

| Parameters | Results |
|---|---|
| Linearity range | 2-10µg/Ml |
| Slope | 11.572 |
| Intercept | 20.823 |
| Regression coefficient ($R^2$) | 0.9991 |

### Precision

### Repeatability

The % RSD for Fluorescence intensity was found to be 0.68%. These results indicate that the method is precise for measurement of DPG. The data for the repeatability of measurement is depicted in Table:5.

**Table 5:** Data for Repeatability for estimation of DPG

| Concentration (µg /mL) | Fluorescence intensity (mean ± SD) (n=7) | %RSD |
|---|---|---|
| 6 | 90.453±0.621 | 0.68 |

### Intermediate precision

The %RSD for intra-day and inter-day precision of DPG was found to be in the range of 0.74-1.34 % and 0.75-1.83 %

respectively. The data for intra-day and inter-day precision for DPG is depicted in Table 6.

**Table 6:** Data for Intermediate precision for estimation of DPG

| Concentration (µg/mL) | Intra-day precision | | Inter-day precision | |
|---|---|---|---|---|
| | Florescence intensity Mean ± SD (n=3) | % RSD | Florescence intensity Mean ± SD (n=3) | % RSD |
| 2 | 42.662±0.317 | 0.74 | 42.553± 0.782 | 1.83 |
| 4 | 68.340±0.596 | 0.87 | 68.647± 0.939 | 1.36 |
| 6 | 91.772±0.701 | 0.76 | 90.472± 0.852 | 0.94 |
| 8 | 113.6317±1.110 | 0.97 | 113.600±1.095 | 0.96 |
| 10 | 134.268±1.802 | 1.34 | 135.853±1.019 | 0.75 |

**Limit of detection and limit of quantitation**

The LOD and LOQ for DPG were found to be 0.15 µg/mL and 0.46 µg/mL respectively. The data for LOD and LOQ of DPG is depicted in Table: 7.

**Table 7:** LOD and LOQ data for estimation of DPG

| Parameters | Results |
|---|---|
| The standard deviation of the Y-intercepts of Calibration curves (n=7) | 0.535 |
| The mean slope of calibration curves (n=7) | 20.798 |
| LOD = 3.3 × (SD/Slope) (µg/mL) | 0.15 |
| LOQ = 10 × (SD/Slope) (µg/mL) | 0.46 |

**Accuracy**

Accuracy was determined in terms of recovery study and the recovery are done at three levels i.e.80%, 100% and 120%. The % recovery of DPG was found to be in the range of 99.40-99.76%. These results indicate that the method is accurate in the measurement of DPG. The data for the accuracy of the method for DPG is depicted in Table: 8.

**Table 8:** Accuracy data of DPG

| Spiked level (%) | DPG amount from sample(mg) | Spiked DPG amount (mg) | Conc. of DPG solution (µg/mL) | Florescence intensity (Mean ± SD) (n=3) | Total amount of drug recovered (mg) | Amount found (mg) | Recovery (%) |
|---|---|---|---|---|---|---|---|
| 80 | 10 | 8 | 5.4 | 83.201±1.24 | 17.96 | 7.95 | 99.39 |
| 100 | 10 | 10 | 6 | 90.693±0.51 | 19.93 | 9.92 | 99.29 |
| 120 | 10 | 12 | 12 | 97.428±1.97 | 22.05 | 12.04 | 100.40 |

**Robustness**

The % RSD for different wavelength and a different scan speed of DPG was found to be in the range of 0.37-.1.47 % and 0.04-0.72% respectively. These results indicate that the method is robust for measurement of DPG. The data for different wavelength and different scan speed for DPG is depicted in Table: 9 and 10

**Table 9:** Robustness data of DPG for different wavelength

| Concentration (µg/mL) | Wavelength (Fluorescence intensity) | | | Fluorescence intensity Mean ± SD | % RSD |
|---|---|---|---|---|---|
| | 275 nm | 277 nm | 279 nm | | |
| 2 | 43.255 | 42.706 | 41.711 | 42.557±0.78 | 1.8 |
| 4 | 69.566 | 68.686 | 67.689 | 68.647±0.93 | 1.36 |
| 6 | 91.231 | 90.636 | 89.55 | 90.472±0.85 | 0.94 |
| 8 | 114.455 | 113.980 | 112.366 | 113.600±1.09 | 0.96 |
| 10 | 136.893 | 135.813 | 134.855 | 135.853±1.01 | 0.75 |

**Table 10:** Robustness data of DPG for different scan speed

| Concentration (µg/mL) | Scan speed (Fluorescence intensity) | | | Fluorescence intensity Mean ± SD | % RSD |
|---|---|---|---|---|---|
| | Fast | Medium | Slow | | |
| 2 | 42.706 | 42.325 | 42.955 | 42.662±0.317 | 0.74 |
| 4 | 68.202 | 68.994 | 67.825 | 68.340±0.596 | 0.87 |
| 6 | 91.023 | 91.88 | 92.413 | 91.772±0.701 | 0.76 |
| 8 | 114.53 | 113.975 | 112.39 | 113.6317±1.110 | 0.97 |
| 10 | 132.287 | 135.813 | 134.704 | 134.268±1.802 | 1.34 |

**Validation Summary**

The summary of validation results is depicted in Table: 11.

**Table 11:** Summary of validation parameters for DPG

| Sr. No. | Parameters | Results |
|---|---|---|
| 1 | Linearity Range | 2-10 µg/ mL |
| 2 | Regression equation | y = 11.572x+20.798 |
| 3 | Regression coefficient($R^2$) | 0.9991 |
| 4 | Precision (%RSD) | |
| | Repeatability (n=7) | 0.68% |
| | Intermediate precision | |
| | Intra-day precision (n=3) | 0.74-1.34% |
| | Inter-day precision (n=3) | 0.75-1.83% |
| 5 | Limit of detection (LOD) | 0.15 µg/ mL |
| 6 | Limit of quantification (LOQ) | 0.46 µg/ mL |
| 7 | Accuracy (n=3) | 99.29-100.40% |
| 8 | Robustness | Robust |

**Analysis of Pharmaceutical dosage form**

Applicability of the proposed method was tested by analysing pharmaceutical dosage form. The percentage of DPG in the pharmaceutical dosage form was calculated from the calibration curve of DPG. The assay value for a pharmaceutical dosage form of DPG was 99.90%. This result is within the range of precise limit. Assay result of a pharmaceutical dosage form of DPG is depicted in Table: 12.

**Table 12:** Analysis of the Pharmaceutical dosage form

| Labelled claim | Fluorescence intensity mean± SD(n=3) | Amount of drug found in the formulation (mg) | Assay (%) |
|---|---|---|---|
| 10 | 55.590±0.93 | 10.019 | 100.19 |

## 4. Conclusion

Spectrofluorimetry method was developed and validated for the Estimation of DPG in the tablet dosage form. The proposed method was found to be specific, accurate, precise

and sensitive. The developed method was applied for the assay of pharmaceutical dosage forms and results were found to be in good agreement with the extract amount.The proposed method can be applied for the routine analysis of tablet dosage form.

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